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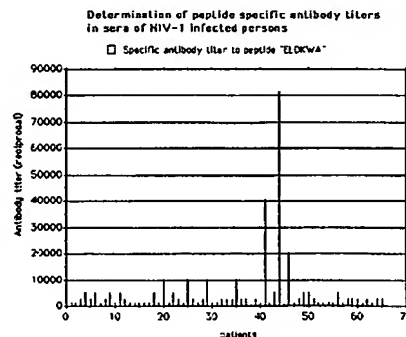
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(54) **Peptides that induce antibodies which neutralize genetically divergent HIV-1 isolates.**

(57) This invention refers to peptides binding to antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and that inhibit the fusion of cells caused by HIV-1. These peptides are applied with an adjuvant, as recombinant fusion proteins, chemically coupled to carrier molecules, as recombinant chimeric viruses or as recombinant antibodies.



The antibody titres were determined by ELISA. The peptide was used in form of a fusionpeptide (in combination with glutathione-S-transferase). The fusionpeptide was coated to 96 microtitre plates (100µl/well) (2.5µg/ml) and incubated over night at 4°C. After washing three times with washing-buffer HIV-1 positive sera were diluted 2<sup>nd</sup> fold (1:40-1:81920) in dilution-buffer and aliquotes were transferred to the test-plate (100µl/well) and incubated for 1h at RT. Then the plates were washed again three times with washing-buffer. As a second antibody goat anti human γ-chain, conjugated with horse radish peroxidase, was used (diluted 1:1000, 100µl/well). After 1h incubation at RT the plates were washed three times with washing-buffer. Then the plates were stained using o-phenylenediamine-dihydrochloride as substrate. The reaction was stopped with 2.5 M H<sub>2</sub>SO<sub>4</sub> and the plates were measured (measuring wavelength 492 nm, reference wavelength 620 nm) and evaluated.

Cutoff = the mean value (4-fold) of a HIV-1 negative serum (1:40) + 3 fold standard deviation. The donors of serum number 20, 25, 29, 35, 41, 44, 46 are HIV-1 positive for at least five years and still asymptomatic.

Figure 2: Graphic of the specific antibody titres to the peptide with the aminoacid sequence "ELDKWA" of 65 sera from HIV-1 positive donors

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This invention refers to peptides that induce antibodies which neutralize genetically divergent HIV-1 isolates. These peptides are applied with an adjuvant, as recombinant fusion proteins, chemically coupled to carrier molecules, as recombinant chimeric viruses or as recombinant antibodies. In addition, the stage of infection can be determined and the progression of the infection can be predicted with these peptides.

## INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) is the late stage clinical manifestation of long term persistent infection with human immunodeficiency virus type 1 (HIV-1). Immune responses directed against the virus and against virus-infected cells during the persistent infection usually fail to mediate resolution to the infection. A possibility to elicit an immune response that can prevent the establishment of a persistent infection or that can prevent the progression to AIDS are vaccines. Most vaccine strategies against HIV-1 are directed against the surface glycoprotein gp160 which is made up of gp120 and gp41 and is responsible for virus binding to the cellular receptor CD4 and fusion activity.

However, in context with gp160 several phenomena that argue against the use of whole gp160 or gp120 as an immunogen were observed. *In vitro* experiments showed, that synergism between HIV-1 gp120 and gp120-specific antibodies block human T cell activation (1). This result supports the hypothesis, that also *in vivo* the humoral immune response against gp120 of HIV-1 suppresses T-cell activation and might be one reason for immunodeficiency. The proposed mechanism for this phenomenon is cross-linking and modulation of CD4 molecules through gp120 and anti-gp120. Experiments from Kion et al.(2) suggest that sequence homologies between gp160 and class II MHC molecules lead to immunodeficiency. In addition, a number of antigenic domains on gp160 are known to induce antibodies that enhance HIV-1 infection (3). Such effects known in context with gp160 could be avoided by using synthetic peptides or other subunit vaccines that only contain immunogenic and neutralizing epitopes as immunogens. Immunogenic peptides corresponding to parts of different viral proteins were already used for successful immunization (4,5,6). The use of synthetic peptides as immunogens offers a number of advantages. The antibodies produced have a predetermined specificity, and in the case of viruses, they can be selected to represent structures on the surface of virions. The synthetic polypeptides also are interesting in that they can induce antibody responses not seen under normal conditions. For example, it was found that in the hemagglutinin of influenza virus there are five major antigenic regions and that under conditions of natural infection the immune response includes antibodies only to these regions. With synthetic polypeptides, an immune response against other regions of the hemagglutinin polypeptide can be generated, and these antibodies have been found to be capable of neutralizing the virus. Therefore it is possible to induce neutralizing antibodies that have a broader reactivity than antibodies induced by whole proteins (4). In addition immunizations with peptides derived from the nucleotide sequence of foot and mouth disease virus (FMDV) are described. In contrast to immunizations with the corresponding whole protein of FMDV, immunizations with these peptides lead to neutralizing antibodies which were also protective (5). Furthermore, a peptide containing part of the V3 loop of gp120 from the HIV-1 isolate HIV-1 IIIb was shown to induce a protective immune response against virus challenge with the same HIV-1 isolate (7,8).

Because synthetic peptides themselves have poor immunogenicity, they have to be coupled to molecules that provide an adjuvant effect such as tetanus toxoid or keyhole limpet hemocyan (5). Another possibility is to clone small peptides as fusion peptides with glutathione S-transferase of *Schistosoma japonicum* (9,10). In addition, attenuated viruses such as vaccinia, polio Sabin type 1 or influenza NA/B-NS can be used as vectors for immunogens. Vaccinia virus is used frequently as a vector of foreign genes of multiple pathogens. For example rabbits inoculated with recombinant vaccinia virus containing sequences from hepatitis B surface antigen (HBsAg), herpes simplex virus glycoprotein D, and influenza virus hemagglutinin produced antibodies to all three foreign antigens (11).

Furthermore, a chimeric polio virus that expressed an epitope from gp41 of HIV-1 induced neutralizing antibodies against gp41 in rabbits (12). Since recently it is also possible to change the genome of influenza virus by *in vitro* mutagenesis (13). By means of this technique it was possible to engineer a stable attenuated influenza A virus (14). In addition by using this technique it was also possible to construct an intertypic chimeric virus, in which a six-amino-acid loop contained in the antigenic site B of the hemagglutinin of an H1 subtype was replaced by the corresponding structures of subtypes H2 and H3 (15). An advantage of influenza virus in this context is the availability of many variants so that repeated vaccination may be possible. Furthermore, influenza virus induces strong secretory and cellular immune responses, which may be advantageous for an anti-HIV-1 vaccine approach. In addition it is unlikely that influenza virus is associated with the development of malignancies. There is no DNA phase involved in the replication of influenza viruses, which excludes the possibility of chromosomal integration of viral influenza genes.

The use of antiidiotypic antibodies is another possibility to achieve a specific immune reaction. Antiidiotypic

antibodies are antibodies that specifically recognize and bind the antigen binding site of another antibody. As the combining sites of antibodies can be structurally looked at as a mirror image of the epitope that is bound, an antiidiotypic antibody corresponds to the mirror image of this primary mirror image, which means that an antiidiotypic antibody displays the internal image of the epitope that is bound by the idiotype antibody. Although one can not always expect to find complete identity between the structure or the amino acid sequence respectively of the antiidiotypic antibody with that of the epitope, one can however see effects in practice that allow the conclusion that there is a structural, sequential or functional similarity between antiidiotypic antibodies and the respective epitopes. The use of antiidiotypic antibodies as a vaccine was initially proposed by Nisonoff and Lamoyi (16). In the case of African Sleeping Disease it was first shown that a protective immune response against the causative agent, *Trypanosoma brucei rhodesiense*, could be elicited in BALB/c mice by vaccinating the mice with antiidiotypic antibody (17). In the case of viral antigens, the formation of antiidiotypic antibodies to a neutralizing epitope on the hemagglutinin molecule of Reovirus Type III was investigated. These antiidiotypic antibodies recognized the cellular receptor of Reovirus-hemagglutinin on both, cytolytic T-cells and neuronal cells, and were able to induce in mice a humoral as well as a cellular immune response specific to Reovirus-hemagglutinin (18, 19, 20).

#### DETAILED DESCRIPTION OF THE INVENTION

Peptides comprising 6 amino acid residues (aa) that bind specifically to the monoclonal antibody 2F5 were used as immunogens to induce neutralizing antibodies against HIV-1. For identification of these peptides overlapping fragments of gp41 (HIV-1 isolate BH10) were cloned as fusion peptides with glutathiontransferase. The different fusion peptides were obtained through hybridization of gp41 corresponding oligonucleotides which were cloned between the Bam HI and the Eco RI site of the plasmid pGEX-2T (Pharmacia). The recombinant plasmids were transformed into the E.coli strain DH5 $\alpha$  and expression of the fusion proteins was induced with isopropylthiogalactoside (IPTG). The E. coli extract was then purified with glutathion-sepharose 4B columns, loaded on sodiumdodecylsulfat-polyacrylamide gels, separated by electrophoresis and protein expression was analyzed by silver staining. Fusion peptides that were reactive with the monoclonal antibody 2F5 were identified by immunoblotting. Using this method peptides which bind to the monoclonal antibody 2F5 were identified: Figure 1 shows Western blots of fusion peptides with overlapping fragments of gp160 of HIV-1 (isolate BH10). In contrast to constructs that comprise aa 597 to 677, 634 to 677 and 648 to 677 (the numbering of amino acid residues corresponds to gp160 of HIV-1 isolate BH10, as described in the Swissprot database entry ENV\$HIV10) which were reactive with the antibody 2F5, a fusion peptide comprising aa 667 to 677 did not show a positive reaction. This was the first indication that the epitope of the monoclonal antibody 2F5 is formed by aa within the sequence from position 648 to 667 of gp160. Based on these results, overlapping 6-mer peptides of this region were fused with the glutathion S-transferase. As shown in figure 1b the peptide containing the aminoacid sequence GLU LEU ASP LYS TRP ALA (aa 662-667) was highly reactive with the antibody 2F5 whereas for peptides containing the aminoacid sequence LEU ASP LYS TRP ALA SER (aa 663-668) or ASP LYS TRP ALA SER LEU (aa 664-669) reactivity with the monoclonal antibody was significantly lower. A peptide containing aminoacid sequence LEU GLU LEU ASP LYS TRP (aa 661-666) showed no reactivity at all. These data suggest that the epitope of the monoclonal antibody comprises the aminoacid sequence GLU LEU ASP LYS TRP ALA that correspond to aa 662-667 on gp160 of the HIV-1 BH10 isolate. In this context both, a synthetic peptide corresponding to this epitope sequence and a fusion protein containing this sequence were able to inhibit neutralization mediated by the 2F5 antibody (Fig.4). Sequence comparison of that region revealed that the corresponding aminoacid sequence is highly conserved between otherwise genetically highly divergent HIV-1 isolates (Table 2a). We also were able to show that fusion peptides with aminoacid substitutions - according to different HIV-1 isolates- in this region were also reactive with the 2F5 antibody (Fig.1c).

The presence of antigenic domains around this region has been reported previously (21,22). Teeuwssen et al. reported of a monoclonal antibody, that reacted with a peptid corresponding to aa 643 to 692 of gp160. In addition Broiden et al. reported that HIV-1 antibody-positive human sera were reactive with a peptide corresponding to region 657-671. However, in both cases a specific epitope was not identified. The monoclonal antibody reported by Teeuwssen et al. had no neutralizing activity. Also the sera reactive with the peptide 657-671 of Broiden et al. showed just partial neutralizing activity. In different neutralization assays this group was able to show neutralizing activity against HIV-1 isolate IIIB but not against SF2 and RF.

In contrast to this result the monoclonal antibody 2F5 neutralizes a variety of different HIV-1 isolates including SF2 and RF (table 1). These data suggest that the antibodies of the sera reported by Broiden et al. as well as the monoclonal antibody reported by Teeuwssen et al. have a different specificity and recognize a different epitope than the antibody 2F5.

The application of the peptides described in the present invention as immunogen has several advantages.

They comprise just 6 aa. Thus other gp160 peptide sequences which induce antibodies that enhance HIV-1 infection or lead to immunosuppression can be avoided (2,3). Furthermore an effective HIV-1 vaccine should induce an immune response against HIV-1 isolates that vary considerably in their genomic sequences. In this context sequence comparison in the region of the 2F5 epitope revealed that the epitope of the 2F5 antibody is highly conserved between different HIV-1 isolates (Table 2a). Since peptides with aa substitutions- corresponding to genetically different HIV-1 isolates- were reactive with the 2F5 antibody (Fig.1c), it is likely that antibodies induced by peptides described in the present invention are directed against a variety of divergent HIV-1 isolates. In addition the 2F5 antibody showed neutralizing activity against a wide variety of genetically different HIV-1 isolates which proves that peptides described in the present invention are presented as neutralizing epitopes (Table 1).

In order to know which variations of the epitope sequence are binding to the monoclonal antibody 2F5 we undertook a peptide mapping with a random hexapeptide library displayed on protein III of a filamentous phage (22a). The hexapeptide sequences of the eluted phage particles were compiled (Table 2b). There is a wide range of variation in the progression of HIV-1 related disease in different HIV-1 infected persons. In many cases HIV-1 infection ends up in AIDS-related complex (ARC) and AIDS within some years, while some HIV-1 positive persons remain asymptomatic. It has been shown that antibody-titers against certain peptide epitopes are much lower in AIDS-patients compared to asymptomatic states (23). We found a significant correlation between the antibody-titers to the peptides described in the present invention and HIV-1 related disease progression (Fig.2), Patients number 20,25,29,35,41,44 and 46 who have a high antibody-titer to peptides described in this invention (Fig.2), did not show any progression in disease within the last five years so far. This means that generation of antibodies induced by peptides described in the present invention can inhibit or at least reduce the progression of HIV-1 related disease. The fact that there are rarely high antibody-titers to peptides described in this invention found in sera of HIV-1 positive patients indicates that these epitopes on gp160 are not recognized readily by the human immune system, resulting in low HIV-1 neutralizing antibody titers specific to these epitopes. An objective of the present invention is also to present the peptides described in the invention in a proper form and to induce a sufficient neutralizing immune response.

#### Example 1:

The cloning and expression of peptides described in the invention as fusion proteins with glutathion S-transferase (GST) and immunizations of mice with these peptides is described. All cloning methods were done according to standard procedures (24). Oligonucleotides corresponding to the peptides described in the invention were hybridized and cloned between the Bam HI and Eco RI site of the plasmid pGEX-2T (Pharmacia). By this the NH<sub>2</sub>-terminal ends of these peptides were fused with the COOH-terminal ends of the GST. In addition, a stop codon was added to the COOH-terminal ends of the gp41 peptide sequences. These constructs were transformed into E. coli DH5 $\alpha$  and expression of the fusion proteins was induced with isopropylthiogalactoside (IPTG). After three hours of induction bacteria were harvested by centrifugation, suspended in phosphate buffered saline (PBS, pH 7.2) containing 1 % Triton-X-100 and sonicated. Bacterial debris were spun down by centrifugation and the supernatant was loaded on glutathion-sepharose 4B columns (Pharmacia). Elution of the fusion proteins was done with 20mM glutathion and 120mM NaCl in 100 mM Tris-HCl (pH 8.0). Purified fusion proteins obtained with this procedure were used for immunizing mice according to standard procedures. As a control, mice were immunized with GST prepared in the same way as the fusion proteins. Sera from mice taken one week after the last immunization showed high neutralizing titers against peptides described in the invention and inhibited HIV-1 replication *in vitro* (Fig.3b and 3c).

#### Example 2:

Example 2 describes the expression of peptide sequences described in the invention as part of the hemagglutinin of influenza A virus. *In vitro* mutagenesis was used to introduce this peptide sequence into the antigenic sites A,B,C,D, and E of the hemagglutinin of influenza A virus (25,26). These chimeric DNA-constructs were then "RNP-transfected" into influenza HK/WSN virus (13). These chimeric influenza/HIV viruses had the antigenic properties of said peptide. In antibody-adsorption experiments these chimeric viruses inhibited HIV-1 neutralization through the antibody 2F5 (Fig.3a). Antisera of mice immunized with the chimeric viruses were reactive with said peptides (Fig.3b). Furthermore, *in vitro* these antisera neutralized different HIV-1 isolates (Fig.3c).

Example 3

Example 3 describes the expression of peptides described in the invention as part of a so called "immunological supermolecule", in where the peptide sequence is inserted into the linker which connects the variable regions of the heavy and light chain of an immunoglobulin molecule. Specifically a single chain Fv construct of a neutralizing anti-HIV-gp120 antibody was made according to standard procedures (27). In this construct peptide sequences described in the invention were inserted into the linker which connects the variable region of the light chain with the variable region of the heavy chain. This recombinant protein was expressed in *E. coli* and purified according to standard procedures. Two functions were observed with this construct. First this construct showed the antigen binding properties of the original antibody and in addition this construct induced, when injected into mice, antibodies that neutralized different HIV-1 isolates (Fig.3c).

This "immunological supermolecule" provides the possibility to obtain an active and passive immunization at the same time.

Basically in such a construct the antigen binding neutralizing properties of an antibody and the presentation of a neutralizing epitope are combined. In already HIV-1 infected persons the progression of infection could be slowed down with the first application by the antigen binding neutralizing properties, before the effective onset of the immunesystem is triggered by the neutralizing epitopes of this molecule. Thus the usual observed "time lag" between immunization and effective immune response of an typical active immunization could be overcome.

In addition it is most likely that during neutralization of already present HIV-1 virions the presentation of the epitope is very efficient.

Example 4

Example 4 describes the formation of antiidiotypic antibodies to antibody 2F5 as well as the production of an antiidiotypic antibody by means of *in vitro* recombination techniques.

Antibody 2F5 was used to immunize mice in order to induce the formation of antiidiotypic antibody. The immunization scheme used was according to standard procedures in order to enhance the frequency at which antiidiotypic antibodies are developed in the animal. The polyclonal sera such obtained were tested for their immunoreactivity, whereby it was determined by means of antigen-competition ELISA that a part of the humoral immune response was indeed directed against the combining site of the antibody 2F5. Thus it was proven that in those sera antiidiotypic antibodies where present. In order to test the concept of vaccination by means of antiidiotypic antibodies, the sera containing antiidiotypic antibodies where subsequently used to immunize another group of mice. After completion of this immunization procedure, it was possible to detect an immune response to the antiidiotypic sera that was qualitatively comparable to the above described immune reaction against the HIV-1 peptide-part of the glutathion-S-transferase fusion protein as described in example 1.

Since it was now proven, that the described peptide has the quality necessary to act as an immunogen, and since furthermore it had been shown that by using antiidiotypic antibodies with internal image quality of the described peptide it was possible to induce a HIV-1 neutralizing immune response, an antiidiotypic antibody was constructed by means of *in vitro* recombination techniques. In order to achieve this goal, one or more hypervariable regions (or parts thereof) of an existing, molecularly cloned antibody where substituted by peptide sequences described in the invention. The respective constructs where expressed as single chain Fv fragments in *E. coli*, and the recombinant proteins where purified according to standard methods. Immunization of mice with the antiidiotypic proteins such produced lead to a HIV-1 neutralising immune response (Fig.3c).

Example 5

Example 5 describes the peptide mapping with a random hexapeptide library and immunizations of mice with phages containing peptides according to SEQ ID NO: 10 through SEQ ID NO: 25. The monoclonal antibody 2F5 was coated onto polystyrol tubes (Maxisorp, Nunc, Denmark) at a concentration of 5 ug/ml in coating buffer (0.1M Na-Carbonate buffer, pH 9.6) overnight at 4°C. After washing with PBS, the surface was blocked with PBS containing 5%w/v skimmed milk powder at 36°C for 2 hours.

Washing with PBS was followed by incubation of a hexapeptide phage display library ( $10^{11}$  transduction units in TPBS (PBS including 0.5% v/v Tween 20) overnight at 5°C. Extensive washing with TPBS was followed by elution of phage with elution buffer (0.1N HCl/Glycine pH 2.2, 1 mg BSA/ml). The eluate is neutralized with 1 M Tris and used for infection of *E. coli* K91Kan. Phage is prepared from the infected culture by the methods described (22a). The procedure is repeated 3 times. The final eluate was used to produce transduced *E. coli* K91Kan. DNA of these clones was sequenced and the respective phage displayed hexapeptide sequence was

derived by computer translation.

Clones with the SEQ ID NO:10 through 25 (Table 2) were used for phage preparation and the respective phages were injected into mice. After two booster injections the respective sera were tested for HIV-1 neutralizing activity. All of these sera were neutralizing *in vitro*. The control serum was produced by immunization with wild-type phage f1 and did not neutralize HIV-1. In addition, oligonucleotides coding for SEQ ID NO: 10 through 25 were introduced into gene VIII of fd-tet between aa 27 and 28 of the unmaturation protein VIII by standard cloning techniques. The recombinant phages were produced in *E. coli* K91Kan, purified by standard techniques (PEG mediated precipitation followed by CsCl gradient centrifugation) and used as immunogen. The respective sera were found to be neutralizing in HIV-1 neutralizing assays whereas anti-wild type fd-tet was not.

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Sequence Listing:

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SEQ ID NO: 1

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

15

TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate BH10

20

POSITION OF THE SEQUENCE IN GP160: from residue 662 to 667

PROPERTIES: Epitope of a human monoclonal antibody directed  
against HIV-1 GP160

25

REFERENCE: translated from GenBank accession M15654,  
nucleotides 7563 to 7580

30

Glu Leu Asp Lys Trp Ala

1 5 6

35

SEQ ID NO: 2

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

40

TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate JS4/26

45

POSITION OF THE SEQUENCE IN GP160: from residue 655 to 660

PROPERTIES: Epitope of a human monoclonal antibody directed  
against HIV-1 GP160

50

REFERENCE: translated from GenBank accession M37576,  
nucleotides 1963 to 1980

55

5     Glu Leu Asn Lys Trp Ala  
      1                    5     6

10

SEQ ID NO: 3

15     LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

20     ORIGIN: GP160 of HIV-1 isolate (patient 3L)

POSITION OF THE SEQUENCE IN GP41: from residue 164 to 169

25     PROPERTIES: Epitope of a human monoclonal antibody directed  
against HIV-1 GP16030     REFERENCE:     translated     from     GenBank     accession     X61352,  
nucleotides 490 to 507

Glu Leu Asp Lys Trp Asp

1                    5     6

35

40     SEQ ID NO: 4

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

45     TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate SF170

POSITION OF THE SEQUENCE IN GP160: from residue 667 to 672

50

PROPERTIES: Epitope of a human monoclonal antibody directed  
against HIV-1 GP16055     REFERENCE:     translated     from     GenBank     accession     M66533,  
nucleotides 1999 to 2016



5   Ala Leu Asp Lys Trp Ala  
     1                   5    6

10

SEQ ID NO: 5

15   LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

20

ORIGIN: GP160 of HIV-1 isolate JH3

POSITION OF THE SEQUENCE IN GP160: from residue 673 to 678

25

PROPERTIES: Epitope of a human monoclonal antibody directed  
against HIV-1 GP160

30

REFERENCE:   translated    from    GenBank    accession    M21138,  
nucleotides 2263 to 2280

35

Gly Leu Asp Lys Trp Ala  
     1                   5    6

40

SEQ ID NO: 6

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

45

TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate Z-84

POSITION OF THE SEQUENCE IN GP160: from residue 669 to 674

50

PROPERTIES: Epitope of a human monoclonal antibody directed  
against HIV-1 GP160

55

5 REFERENCE: translated from GenBank accession J03653,  
nucleotides 2037 to 2054

Gln Leu Asp Lys Trp Ala  
10 1 5 6

15 SEQ ID NO: 7

LENGTH OF SEQUENCE: 6 amino acid residues  
TYPE OF SEQUENCE: amino acid sequence  
20 TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate CAM1 proviral genome  
25 POSITION OF THE SEQUENCE IN GP160: from residue 662 to 667

PROPERTIES: Epitope of a human monoclonal antibody directed  
against HIV-1 GP160  
30

REFERENCE: translated from GenBank accession D10112,  
nucleotides 8209 to 8226

35 Glu Leu Asp Thr Trp Ala  
1 5 6

40

SEQ ID NO: 8

45 LENGTH OF SEQUENCE: 6 amino acid residues  
TYPE OF SEQUENCE: amino acid sequence  
TYPE OF FRAGMENT: inner fragment

50 ORIGIN: GP160 of HIV-1 isolate JS4/6  
POSITION OF THE SEQUENCE IN GP160: from residue 659 to 664

55 PROPERTIES: Epitope of a human monoclonal antibody directed  
against HIV-1 GP160

5 REFERENCE: translated from GenBank accession M37491,  
nucleotides 2416 to 2433

10 Ala Leu Asp Thr Trp Ala  
1 5 6

15 SEQ ID NO: 9

LENGTH OF SEQUENCE: 6 amino acid residues

20 TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

25 ORIGIN: GP160 of HIV-1 isolate SBB

POSITION OF THE SEQUENCE IN GP160: from residue 413 to 418  
(partial sequence)

30 PROPERTIES: Epitope of a human monoclonal antibody directed  
against HIV-1 GP160

35 REFERENCE: translated from GenBank accession M77229,  
nucleotides 1239 to 1256

40 Lys Leu Asp Glu Trp Ala  
1 5 6

45 SEQ ID NO: 10

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

50 TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage FUSE5

55 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

5

Ser Leu Asp Lys Trp Ala

1                      5    6

10

SEQ ID NO: 11

LENGTH OF SEQUENCE: 6 amino acid residues

15

TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

20

ORIGIN: p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

25

Gly Arg Asp Lys Trp Ala

30

1                      5    6

SEQ ID NO: 12

35

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

40

ORIGIN: p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

45

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

50

Gly Ala Asp Lys Trp Ala

1                      5    6

55

SEQ ID NO: 13

LENGTH OF SEQUENCE: 6 amino acid residues

Gly Ala Asp Lys Trp Gly

1 5 6

5

SEQ ID NO: 16

10 LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

15

ORIGIN: p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

20

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

25

Gly Ala Asp Lys Trp Asn

1 5 6

30

SEQ ID NO: 17

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

35

TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5

40

POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

45

Gly Ala Asp Lys Trp Cys

1 5 6

50

SEQ ID NO: 18

55 LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5

5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

10

Gly Ala Asp Lys Trp Val

1                      5    6

15

SEQ ID NO: 19

LENGTH OF SEQUENCE: 6 amino acid residues

20

TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

25

ORIGIN: p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

30

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

Gly Ala Asp Lys Trp His

35

1                      5    6

SEQ ID NO: 20

40

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

45

ORIGIN: p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

50

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

55

Gly Ala Asp Lys Cys His

1                      5    6

SEQ ID NO: 21

5

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

10

ORIGIN: p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

15

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

20

Gly Ala Asp Lys Cys Gln

1                      5    6

25

SEQ ID NO: 22

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

30

TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5

35

POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

40

Ala Tyr Asp Lys Trp Ser

1                      5    6

45

SEQ ID NO: 23

50

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

55

ORIGIN: p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

5

Ala Phe Asp Lys Trp Val

1                      5      6

10

SEQ ID NO: 24

15

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

20

ORIGIN: p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

25

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

30

Gly Pro Asp Lys Trp Gly

1                      5      6

35

SEQ ID NO: 25

40

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: inner fragment

45

ORIGIN: p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

50

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

55

Ala Arg Asp Lys Trp Ala

1                      5      6



SEQ ID NO: 26

5      LENGTH of SEQUENCE: 18 base pairs  
TYPE OF SEQUENCE: nucleotide sequence

10     TYPE OF STRAND: single strand  
TOPOLOGY OF SEQUENCE: linear  
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA  
TYPE OF FRAGMENT: inner fragment

15     ORIGIN: GP160 of HIV-1 isolate BH10  
REFERENCE: GenBank accession M15654  
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 7564-7580

20

gaattagata aatgggca  
18  
25     1            11

30      SEQ ID NO: 27

30      LENGTH of SEQUENCE: 18 base pairs  
TYPE OF SEQUENCE: nucleotide sequence

35     TYPE OF STRAND: single strand  
TOPOLOGY OF SEQUENCE: linear  
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA

40     TYPE OF FRAGMENT: inner fragment  
ORIGIN: GP160 of HIV-1 isolate JS4/26  
REFERENCE: GenBank accession M37576

45     POSITION OF THE SEQUENCE IN DATABASE ENTRY: 1963-1980

50     gaattgaata agtgggca  
18  
1            11

55

SEQ ID NO: 28

LENGTH of SEQUENCE: 18 base pairs  
TYPE OF SEQUENCE: nucleotide sequence

5

TYPE OF STRAND: single strand  
TOPOLOGY OF SEQUENCE: linear  
10 TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA  
TYPE OF FRAGMENT: inner fragment  
ORIGIN: GP160 of HIV-1 isolate (patient 3L)  
15 REFERENCE: GenBank accession X61352  
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 490-507

20

gaattagata agtgggac  
18

25

1 11

30

SEQ ID NO: 29

LENGTH of SEQUENCE: 18 base pairs  
TYPE OF SEQUENCE: nucleotide sequence

35

TYPE OF STRAND: single strand  
TOPOLOGY OF SEQUENCE: linear  
40 TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA  
TYPE OF FRAGMENT: inner fragment  
ORIGIN: GP160 of HIV-1 isolate SF170  
REFERENCE: GenBank accession M66533  
45 POSITION OF THE SEQUENCE IN DATABASE ENTRY: 1999-2016

50

gcattggaca agtgggca  
18

55

1 11

SEQ ID NO: 32

5      LENGTH of SEQUENCE: 18 base pairs  
TYPE OF SEQUENCE: nucleotide sequence

10     TYPE OF STRAND: single strand  
TOPOLOGY OF SEQUENCE: linear  
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA  
TYPE OF FRAGMENT: inner fragment

15     ORIGIN: GP160 of HIV-1 isolate CAM1 proviral genome  
REFERENCE: GenBank accession D10112  
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 8209-8226

20

25     gaattggata cgtgggca  
18  
1                11

30

SEQ ID NO: 33

35     LENGTH of SEQUENCE: 18 base pairs  
TYPE OF SEQUENCE: nucleotide sequence

40     TYPE OF STRAND: single strand  
TOPOLOGY OF SEQUENCE: linear  
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA  
TYPE OF FRAGMENT: inner fragment

45     ORIGIN: GP160 of HIV-1 isolate JS4/6  
REFERENCE: GenBank accession M37491  
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 2416-2433

50

55     gcattggata cgtgggca  
18  
1                11

5 SEQ ID NO: 34

LENGTH of SEQUENCE: 18 base pairs

10 TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand

TOPOLOGY OF SEQUENCE: linear

15 TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA

TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate SBB

REFERENCE: GenBank accession M77229

20 POSITION OF THE SEQUENCE IN DATABASE ENTRY: 1239-1256

25 aagttagatg agtgggca

18

1 11

30

SEQ ID NO: 35

35 LENGTH of SEQUENCE: 18 base pairs

TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand

40 TOPOLOGY OF SEQUENCE: linear

TYPE OF SEQUENCED MOLECULE: phage DNA

TYPE OF FRAGMENT: inner fragment

45 ORIGIN: gene coding for p3 fusion protein of filamentous phage  
FUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

50

55

5   tcgcttgata agtgggcc  
    18  
    1           11

10   SEQ ID NO: 36

    LENGTH of SEQUENCE: 18 base pairs

15   TYPE OF SEQUENCE: nucleotide sequence

    TYPE OF STRAND: single strand

    TOPOLOGY OF SEQUENCE: linear

20   TYPE OF SEQUENCED MOLECULE: phage DNA

    TYPE OF FRAGMENT: inner fragment

    ORIGIN: gene coding for p3 fusion protein of filamentous phage  
    FUSE5

25

    POSITION OF THE SEQUENCE IN p3: 10 to 27

30

    gggcgtgata agtgggcg  
    18  
35   1           11

    SEQ ID NO: 37

40

    LENGTH of SEQUENCE: 18 base pairs

    TYPE OF SEQUENCE: nucleotide sequence

45   TYPE OF STRAND: single strand

    TOPOLOGY OF SEQUENCE: linear

    TYPE OF SEQUENCED MOLECULE: phage DNA

    TYPE OF FRAGMENT: inner fragment

50   ORIGIN: gene coding for p3 fusion protein of filamentous phage  
    FUSE5

55

    POSITION OF THE SEQUENCE IN p3: 10 to 27

5 ggggctgata agtgggcg  
18  
1 11

10 SEQ ID NO: 38

LENGTH of SEQUENCE: 18 base pairs  
TYPE OF SEQUENCE: nucleotide sequence

15

TYPE OF STRAND: single strand

TOPOLOGY OF SEQUENCE: linear

TYPE OF SEQUENCED MOLECULE: phage DNA

20

TYPE OF FRAGMENT: inner fragment

ORIGIN: gene coding for p3 fusion protein of filamentous phage  
FUSE5

25

POSITION OF THE SEQUENCE IN p3: 10 to 27

30

gctcatgaaa agtgggcg  
18  
1 11

35

SEQ ID NO: 39

40 LENGTH of SEQUENCE: 18 base pairs

TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand

45

TOPOLOGY OF SEQUENCE: linear

TYPE OF SEQUENCED MOLECULE: phage DNA

TYPE OF FRAGMENT: inner fragment

50

ORIGIN: gene coding for p3 fusion protein of filamentous phage  
FUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

55

5 gcttgtgatac agtgggacg

18

1 11

10 SEQ ID NO: 40

LENGTH of SEQUENCE: 18 base pairs

15 TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand

TOPOLOGY OF SEQUENCE: linear

20 TYPE OF SEQUENCED MOLECULE: phage DNA

TYPE OF FRAGMENT: inner fragment

ORIGIN: gene coding for p3 fusion protein of filamentous phage  
FUSE5

25

POSITION OF THE SEQUENCE IN p3: 10 to 27

30

ggagctgata agtggggc

18

35 1 11

SEQ ID NO: 41

40 LENGTH of SEQUENCE: 18 base pairs

TYPE OF SEQUENCE: nucleotide sequence

45 TYPE OF STRAND: single strand

TOPOLOGY OF SEQUENCE: linear

TYPE OF SEQUENCED MOLECULE: phage DNA

TYPE OF FRAGMENT: inner fragment

50 ORIGIN: gene coding for p3 fusion protein of filamentous phage  
FUSE5

55

POSITION OF THE SEQUENCE IN p3: 10 to 27

5      ggagctgata agtggaat  
      18  
      1            11

10     SEQ ID NO: 42

      LENGTH of SEQUENCE: 18 base pairs  
      TYPE OF SEQUENCE: nucleotide sequence

15

      TYPE OF STRAND: single strand  
      TOPOLOGY OF SEQUENCE: linear  
      TYPE OF SEQUENCED MOLECULE: phage DNA

20

      TYPE OF FRAGMENT: inner fragment  
      ORIGIN: gene coding for p3 fusion protein of filamentous phage  
      FUSE5

25

      POSITION OF THE SEQUENCE IN p3: 10 to 27

30

      ggcgctgata aatggtgt  
      18  
      1            11

35

      SEQ ID NO: 43

40

      LENGTH of SEQUENCE: 18 base pairs  
      TYPE OF SEQUENCE: nucleotide sequence

45

      TYPE OF STRAND: single strand  
      TOPOLOGY OF SEQUENCE: linear  
      TYPE OF SEQUENCED MOLECULE: phage DNA  
      TYPE OF FRAGMENT: inner fragment

50

      ORIGIN: gene coding for p3 fusion protein of filamentous phage  
      FUSE5

55



5 POSITION OF THE SEQUENCE IN p3: 10 to 27

10 ggcgctgata aatgggtt  
18  
1 11

15 SEQ ID NO: 44

LENGTH of SEQUENCE: 18 base pairs

20 TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand

TOPOLOGY OF SEQUENCE: linear

25 TYPE OF SEQUENCED MOLECULE: phage DNA

TYPE OF FRAGMENT: inner fragment

ORIGIN: gene coding for p3 fusion protein of filamentous phage  
FUSE5

30

POSITION OF THE SEQUENCE IN p3: 10 to 27

35

ggggctgata agtggcat  
18  
40 1 11

SEQ ID NO: 45

45 LENGTH of SEQUENCE: 18 base pairs

TYPE OF SEQUENCE: nucleotide sequence

50 TYPE OF STRAND: single strand

TOPOLOGY OF SEQUENCE: linear

TYPE OF SEQUENCED MOLECULE: phage DNA

TYPE OF FRAGMENT: inner fragment

55 ORIGIN: gene coding for p3 fusion protein of filamentous phage  
FUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

5

10 ggagctgata aatgtcat  
18  
1 11

15 SEQ ID NO: 46

LENGTH of SEQUENCE: 18 base pairs

TYPE OF SEQUENCE: nucleotide sequence

20

TYPE OF STRAND: single strand

TOPOLOGY OF SEQUENCE: linear

25 TYPE OF SEQUENCED MOLECULE: phage DNA

TYPE OF FRAGMENT: inner fragment

ORIGIN: gene coding for p3 fusion protein of filamentous phage  
FUSE5

30

POSITION OF THE SEQUENCE IN p3: 10 to 27

35

ggagctgata aatgtcag  
18  
40 1 11

SEQ ID NO: 47

45

LENGTH of SEQUENCE: 18 base pairs

TYPE OF SEQUENCE: nucleotide sequence

50

TYPE OF STRAND: single strand

TOPOLOGY OF SEQUENCE: linear

TYPE OF SEQUENCED MOLECULE: phage DNA

TYPE OF FRAGMENT: inner fragment

55

5 ORIGIN: gene coding for p3 fusion protein of filamentous phage  
fUSE5

10 POSITION OF THE SEQUENCE IN p3: 10 to 27

15 gcttatgata agtggagt  
18  
1 11

20 SEQ ID NO: 48

LENGTH of SEQUENCE: 18 base pairs  
TYPE OF SEQUENCE: nucleotide sequence

25 TYPE OF STRAND: single strand  
TOPOLOGY OF SEQUENCE: linear  
TYPE OF SEQUENCED MOLECULE: phage DNA  
30 TYPE OF FRAGMENT: inner fragment  
ORIGIN: gene coding for p3 fusion protein of filamentous phage  
fUSE5

35 POSITION OF THE SEQUENCE IN p3: 10 to 27

40 gcttttgata agtgggtt  
18  
1 11

45 SEQ ID NO: 49

50 LENGTH of SEQUENCE: 18 base pairs  
TYPE OF SEQUENCE: nucleotide sequence

55 TYPE OF STRAND: single strand  
TOPOLOGY OF SEQUENCE: linear  
TYPE OF SEQUENCED MOLECULE: phage DNA

5 TYPE OF FRAGMENT: inner fragment  
 ORIGIN: gene coding for p3 fusion protein of filamentous phage  
 FUSE5

10 POSITION OF THE SEQUENCE IN p3: 10 to 27

15 gggcctgata aatgggggt

18

1 11

20

SEQ ID NO: 50

25 LENGTH of SEQUENCE: 18 base pairs  
 TYPE OF SEQUENCE: nucleotide sequence

30 TYPE OF STRAND: single strand  
 TOPOLOGY OF SEQUENCE: linear  
 TYPE OF SEQUENCED MOLECULE: phage DNA  
 TYPE OF FRAGMENT: inner fragment

35 ORIGIN: gene coding for p3 fusion protein of filamentous phage  
 FUSE5

40 POSITION OF THE SEQUENCE IN p3: 10 to 27

40

45 gctcgtgata agtgggcg

18

1 11

50

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**Figure legends:**

**Fig.1:** Western blots of fusion peptides. Recombinant proteins expressed in *E. coli* were purified as described in example 1 and 100ng of each fusion peptide was fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on a 20% polyacrylamidegel and electroblotted onto a nitrocellulose filter. The blots were blocked with 0.5% nonfat dried milk in phosphate-buffered saline containing 0.1% Tween for 1h at room temperature. After washing, blots were incubated with antibody 2F5 (500ng/ml) for 1h at room temperature. After washing, blots were incubated for 1 h at room temperature with anti-human IgG-alkaline-phosphatase-conjugat. Blots were developed with 1M diethanolaminbuffer(pH9.6) containing 350ug/ml nitro-blue tetrazolium chloride and 350 ug/ml 5-bromo-4-chloro-3-indolyl-phosphate.

**Fig.1a:** Lane 1, glutathion S-transferase (GST); lane 2, amino acids (aa) 597-677 of gp160 fused with GST; lane 3, aa 634-677 fused with GST; lane 4, aa 648-677 fused with GST; lane 5 aa 667-677 fused with GST.

**Fig.1b:** lane 1, GST; lane 2, GST fused with aa GLU LEU ASP LYS TRP ALA (aa 662-667); lane 3, GST with aa LEU ASP LYS TRP ALA SER (aa 663-668); lane 4, GST with ASP LYS TRP ALA SER LEU (aa 664-669); lane 5, GST with aa LEU GLU LEU ASP LYS TRP (aa 661-666)

**Fig.1c:** Fusionpeptides with amino acid substitutions according to HIV-1 isolates with differences in the region of the 2F5 epitope. Amino acid differences are underlined. Lane 1, GST; lane 2, GLU LEU ASP LYS TRP ALA; lane 3, GLN LEU ASP LYS TRP ALA; lane 4, GLY LEU ASP LYS TRP ALA; lane 5, ALA LEU ASP LYS TRP ALA; lane 6, GLU LEU ASN LYS TRP ALA (reaction of this fusion peptide with the 2F5 antibody is not visible in this Western blot; however in ELISA competition assays this peptide was competitive to recombinant gp41 for binding to the 2F5 antibody); lane 7, GLU LEU ASP THR TRP ALA; lane 8, GLU LEU ASP LYS TRP ASP

**Claims**

1. Peptides binding to antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and that inhibit the fusion of cells caused by HIV-1, characterized in that said peptides are composed according to one of the SEQ ID NO: 1 through SEQ ID NO: 25
2. Peptides according to claim 1, characterized in that they are genetically encoded by the nucleotide sequence according to one of the SEQ ID NO: 2 or by sequences hybridising to said sequence or by sequences that are deduced from SEQ ID NO: 26 through SEQ ID NO: 50 by degeneration.
3. Peptides according to claim 1, which upon injection into a mammal either alone or in combination with an adjuvant cause an immune response that leads to the generation of HIV-1 neutralizing antibodies.
4. Peptides according to claim 3, whereby adjuvant is a substance to which said peptides are bound through chemical interaction.
5. Peptides according to claims 1 and 3 being part of fusion peptides, characterized in that a molecule is used as adjuvant to which said peptides are bound by fusion of the respective nucleotide sequences and subsequent expression of the fusion genes in a biological expression system.
6. Peptides according to claims 1 through 5, characterized in that they are used to select antibodies or antibody fragments binding to HIV-1 *in vitro*.
7. Peptides according to claims 1 through 6, characterized in that they are used in an immunological test to determine the neutralization titer in complete sera of patients or experimental animals infected with HIV-1, or to determine the status of infection or to make a prognosis on the further progress of infection.
8. Fusion peptides according to claim 5, characterized in that one or more peptides according to one of the SEQ ID NO: 1 through SEQ ID NO: 25 are used as linker or as part thereof in order to link the variable domains of a single chain Fv fragment.
9. Fusion peptides according to claim 5, characterized in that one or more peptides according to one of the SEQ ID NO: 1 through SEQ ID NO: 25 substitute one or more parts of the peptide sequence of a monoclonal antibody.

10. Fusion peptides according to claims 5 and 9, characterized in that one or more peptides according to one of the SEQ ID NO: 1 through SEQ ID NO: 25 are expressed as part of one or more hypervariable regions of a monoclonal antibody.
- 5 11. Fusion peptides according to claims 5, 9 or 10, characterized in that they are either expressed, or chemically or enzymatically synthesized as part of a single chain Fv fragment or as part of a Fab fragment.
12. Fusion peptides according to claim 5, characterized in that one or more peptides according to one of the SEQ ID NO: 1 through SEQ ID NO:25 substitute one or more parts of the peptide sequence of a viral protein, or are inserted into antigenic sites of a viral protein.
- 10 13. Fusion peptides according to claim 12, characterized in that they are part of a virus.
14. Fusion peptides according to claims 12 or 13, characterized in that the viral protein is the hemagglutinin or neuraminidase of influenza virus.
- 15 15. Fusion peptides according to claims 12 or 13, characterized in that the viral protein is the surface antigen or the core antigen of hepatitis B virus.
- 20
- 25
- 30
- 35
- 40
- 45
- 50
- 55

5 Tables10 a) *in vitro* neutralization assays:

	IIIB	MN	Isolate		A	C
			RF	SF2		
number of						
15 positive tests	8/8	2/2	2/2	n.t.	8/8	4/4
neutralizing concentration ( $\bar{I}g/ml$ )						
	10	10	10		50	10

## 20 b) syncytia inhibition assay:

	IIIB	MN	Isolate		A	C
			RF	SF2		
25 number of						
positive tests	18/18	11/11	6/10	1/1	1/1	2/3
30 EC <sub>50</sub> ( $\bar{I}g/ml$ )	12,8	12	13,7	1,9	27	10

## 35 Table 1: Neutralizing properties of human monoclonal antibody 2F5

40 a) *in vitro* neutralization assay: Different concentrations of the 2F5 antibody were incubated with cellfree virus preparations ( $10^2$ - $10^3$  TCID<sub>50</sub>) 1 h at 37°C. Aliquots of  $10^5$  H9 cells were added to virus/antibody mixtures and incubated for an additional hour at 37°C. After 20 days p24 antigen

45 concentration as indicator for virus replication was determined from supernatants according to standard procedures.

b) Syncytia inhibition assay: Antibody/virus mixtures were prepared as described in table 1a. To this mixtures  $10^5$  AA2

50 cells were added and incubated at 37°C. After 5 days syncytia formation as indicator for HIV-1 replication was evaluated.

Abbreviations: A and C are clinical isolates from Vienna

55



5  
 |E|L|D|K|W|A| 43 a)  
 |A|:|:|:|:| 5  
 |:|:|N|:|:| 1  
 10 |:|:|:|:|D| 2  
 |A|:|:|T|:|:| 3  
 |Q|:|:|:|:| 2  
 |:|:|:|T|:|:| 1  
 15 |G|:|:|:|:| 1  
K	:	:	E	:	:	1
20	S	:	:	:	:	:
G	R	:	:	:	:	
G	A	:	:	:	:	
A	H	E	:	:	:	
25	A	C	:	Q	:	:
G	A	:	:	:	G	
G	A	:	:	:	N	
G	A	:	:	:	C	
30	G	A	:	:	:	V
G	A	:	:	:	H	
G	A	:	:	C	H	
G	A	:	:	C	Q	
35	A	Y	:	:	:	S
A	F	:	:	:	V	
G	P	:	:	:	G	
A	R	:	:	:	A	
 40

Table 2: Peptide sequences bound by antibody 2F5:

45 a: sequences present on gp160 of different HIV-1 isolates. The number on the right side of each sequence indicates the number of incidences in the databases that were screened (SwissProt and GenPept).  
 50

b: binding sequences found by screening a random hexapeptide library expressed on the surface of filamentous phage (sequences already described in a) are not included).  
 55

Abb.1a

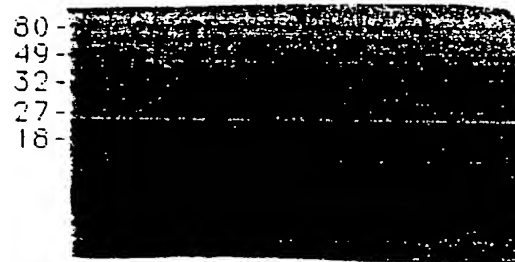
KD



M 1 2 3 4 5

Abb.1b

KD



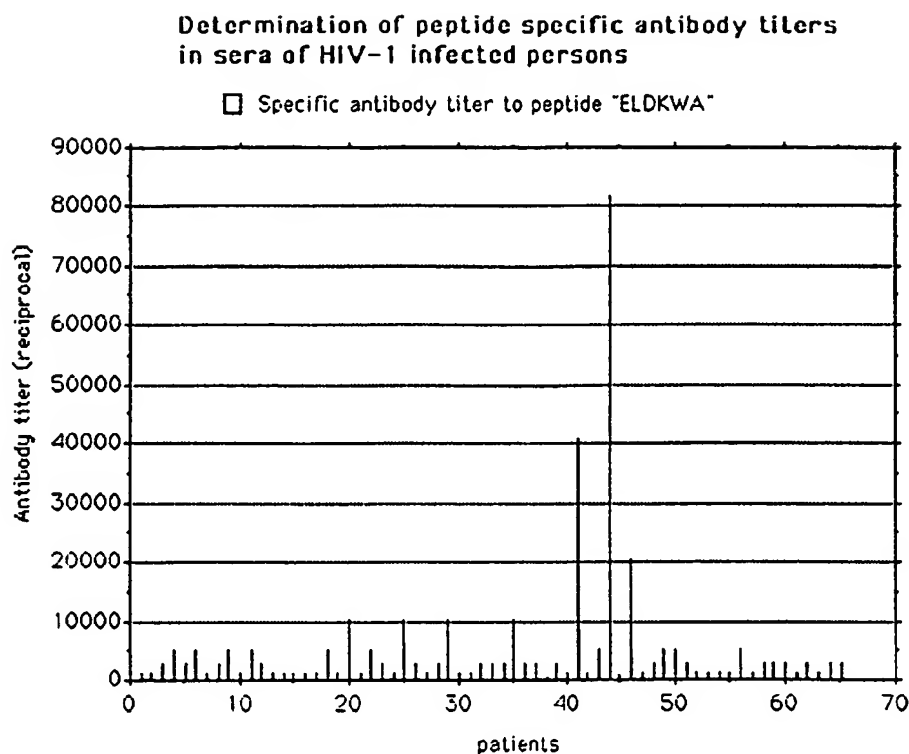
M 1 2 3 4 5

Abb.1c

KD



1 2 3 4 5 6 7 8



The antibody titres were determined by ELISA. The peptide was used in form of a fusionpeptide (in combination with glutathione-S-transferase). The fusionpeptide was coated to 96 microtitre plates 100µl/well (2.5µg/ml) and incubated over night at 4°C. After washing three times with washing-buffer HIV-1 positive sera were diluted 2<sup>n</sup> fold (1:40-1:81920) in dilution-buffer and aliquotes were transferred to the test-plate (100µl/well) and incubated for 1h at RT. Then the plates were washed again three times with washing-buffer. As a second antibody goat anti human γ-chain, conjugated with horse radish peroxidase, was used (diluted 1:1000, 100µl/well). After 1h incubation at RT the plates were washed three times with washing-buffer. Then the plates were stained using o-phenylene-diamine-dihydrochloride as substrate. The reaction was stopped with 2.5 M H<sub>2</sub>SO<sub>4</sub> and the plates were measured (measure wavelength 492 nm, reference wavelength 620 nm) and evaluated.

Cutoff = the mean value (4-fold) of a HIV-1 negative serum (1:40) + 3 fold standard deviation. The donors of serum number 20, 25, 29, 35, 41, 44, 46 are HIV-1 positive for at least five years and still asymptomatic.

**Figure 2:** Graphic of the specific antibody titres to the peptide with the aminoacid sequence "ELDKWA" of 65 sera from HIV-1 positive donors

### Influenza/HIV inhibition of HIV-1 IIIB neutralization

monoklonal antibodies:	residual IIIB neutralization titer in % after incubation with:		
	Mock	Influenza WSN	Influenza/HIV
2F5	100%	100%	10%
2G12	100%	100%	100%

Figure 3a: Influenza/HIV inhibition of HIV-1 neutralization. Results are expressed as reciprocal of the serum dilution giving > 90% reduction in HIV titer following preincubation of the mABs 2F5 and 2G12 with culture medium (Mock), influenza WSN or influenza/HIV. 2F5 is the monoclonal antibody specific for the different epitope on gp160. Residual HIV-neutralizing activity was determined by incubating dilutions of the antibody/virus mixture with  $10^3$  infectious units (TCID<sub>50</sub>) of HIV-1 IIIB for 1h at 37°C. Aliquots (100µl) of medium containing  $10^4$  C8166 cells were added and the presence of syncytia recorded after 48h as an indication of HIV infection.

Immunogen:	Antibodytiter:
K1	< 10
K2	< 10
K3	< 10
F1	1600
F2	6400
F3	800
SM1	3200
SM2	400
SM3	800
V1	3200
V2	3200
V3	6400
rA1	< 10
rA2	400
rA3	800

Figure 3b: Antibodytiter. Three Balb/c mice each were immunized with either 100µg GST (K), 100µg Fusion protein (F), 100µg of the "immunological supermolecule", 100µg of the recombinant antiidiotypic antibody (rA) or 4.0 log<sub>10</sub>TCID<sub>50</sub> of the recombinant influenza/HIV virus (V) and were boosted after 2 and 4 weeks. One week after the last immunization ELISA antibody were determined. Results are given as reciprocal values that gave significant positive values. The cutoff was the double value of a normal mouse serum. Recombinant gp41 was used as an antigen.

## Reciprocal neutralization titers of HIV-1 isolates

Antiserum	IIIB	RF	MN
P1	40	40	40
P2	80	40	40
P3	40	20	20
V1	40	80	40
V2	20	40	<10
V3	160	80	80
SM1	20	40	40
SM2	40	80	80
SM3	<10	<10	<10
rA1	40	40	40
rA2	40	80	20
rA3	80	40	40

Figure 3c: Neutralization of HIV-1 infection. Neutralization titers were determined by incubating 10 $\mu$ l of heat inactivated antiserum with 40 $\mu$ l virus supernatant containing 10<sup>3</sup> infectious units of HIV-1 at 37°C for 1h. Residual HIV-1 infectivity was measured as described in fig. 3a. Abbreviations: P ... fusionpeptide, V ... chimeric influenza/HIV virus, SM ... "immunological supernomolecule", rA ... recombinant antibody. Reciprocal neutralization titers of all controls were lower than 10.

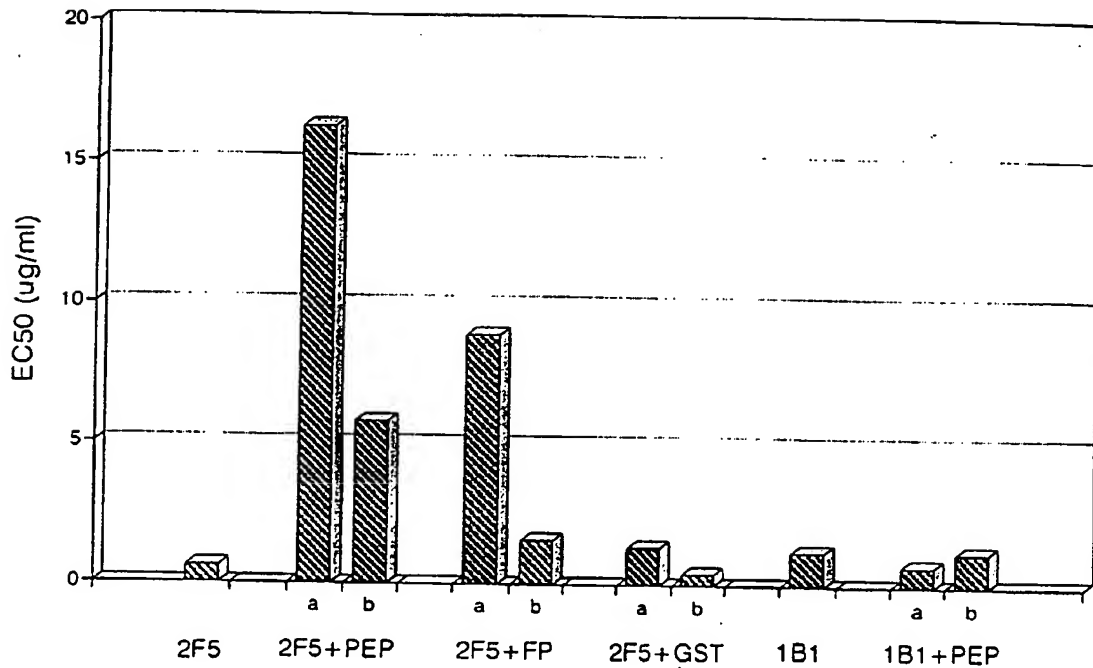


Fig. 4: Inhibition of neutralization by peptides. Synthetic peptide (PEP), fusion peptide (FP), and glutathion-S-transferase (GST) were preincubated with humAb 2F5 or 1B1 for 1 h at 37 °C, and then a syncytia inhibition assay was performed. Antibodies were diluted in 2-fold steps starting with 5 µg/well. PEP, synthetic peptide ELDKWA (peptide corresponding to SEQ ID NO: 1): a 25 µg, b 5 µg per well; FP, fusion peptide ELDKWA with GST: a 25 µg, b 5 µg per well; GST, glutathion-S-transferase: a 25 µg, b 5 µg per well; 1B1, neutralizing anti gp120 humAb